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Human papillomavirus serology and the risk of esophageal and gastric cancers: Results from a cohort in a high-risk region in China

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Each year, esophageal and gastric cancers cause more than 900,000 deaths worldwide. Human papilloma virus (HPV), especially type 16, has been suggested to have a role in the etiology of esophageal cancer, however, the results of previous seroepidemiological studies have not been consistent. We conducted a large prospective study to examine the association between serum antibodies to HPV 16, HPV 18 and HPV 73 and subsequent development of esophageal squamous cell carcinoma (ESCC), gastric cardia adenocarcinoma (GCA), and gastric noncardia adenocarcinoma (GNCA) in a high-risk population for these cancers in Linxian, China. Case and control subjects for this study were selected from the 29,584 participants of the Linxian General Population Trial. Prediagnostic serum samples from 99 cases of ESCC, 100 cases of GCA, 70 cases of GNCA, and 381 age- and sex- matched controls were selected for this study. The presence of antibodies to HPV virus-like particles was determined by type-specific enzyme-linked immunosorbent assays. Fewer than 15% of ESCC, GCA, or GNCA cases were positive for each HPV type, and no significant associations were found. The adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for HPV 16 seropositivity and ESCC, GCA, and GNCA risk were 1.6 (0.8–3.3), 1.3 (0.6–2.8) and 0.4 (0.1–1.6), respectively. The comparable ORs (95% CIs) for HPV 18 were 1.0 (0.4–2.2), 0.9 (0.4–2.1) and 1.5 (0.6–3.4). For HPV 73, these figures were 1.3 (0.6–2.5), 1.2 (0.6–2.3) and 0.9 (0.4–2.1). The results of this study do not support a major role for HPV 16, HPV 18 and HPV 73 in the etiology of esophageal and gastric cancers in Linxian, China.

Key words: esophageal cancer; human papillomavirus; serology

Oncogenic types of human papillomavirus (HPV), most notably HPV 16 and HPV 18, are recognized as the most significant risk factors for cervical cancer.[1] The role of HPV in the etiology of cancers of vulva, anus, penis and oropharyngeal cavity has also been established.[2]

The role of HPV in the etiology of esophageal cancer, however, remains controversial. Syrjanen first suggested a role for HPV in the etiology of esophageal squamous cell carcinoma (ESCC) in 1982, based on observing characteristic histological findings suggesting the presence of HPV in benign esophageal epithelia and malignant esophageal tumors.[3] During the past 20 years, several studies have used a variety of techniques, including detection of HPV DNA in esophageal tumor tissues and serologic methods, to examine the association between exposure to HPV and risk of ESCC.[4] The results of HPV DNA studies have not been consistent: case series using polymerase chain reaction (PCR) have found evidence for the presence of HPV in the tumor tissues varying from 0 to 67%.[4]

Type-restricted serologic assays for different HPV types using virus-like particles were first developed in the mid-1990s,[5] and were subsequently shown to be relatively specific for each HPV type.[6] Seroreactivity to HPV 16 is a strong marker of continuous past exposure to HPV 16 and is associated with squamous intraepithelial lesions of the uterine cervix.[6,7] So far 5 seroepidemiological studies, 2 prospective[8,9] and 3 retrospective (case–control),[10–12] have examined the association of anti-HPV 16 IgG antibodies with the risk of ESCC. Both prospective studies found a strong positive association between HPV 16 and ESCC. Dillner *et al*. analyzed data from 29 cases of ESCC and found an odds ratio (OR) and 95% confidence interval (95% CI) of 12 (2.0–123).[8] Bjorge *et al*. studied 41 cases of ESCC and found an OR (95% CI) of 10 (1.0–510) for the same association.[9] The results of 1 of the retrospective serologic studies also showed a significant 4.5-fold increase in the risk of ESCC associated with seropositivity to HPV 16,[10] but the 2 other studies did not show any increased risk.[11,12] Two of these serologic studies, 1 prospective and 1 retrospective, also examined the relationship of HPV 18 seropositivity and ESCC, but found no significant association.[9,11] HPV 73 has been reported to be the most common type of HPV found in benign and malignant esophageal tumors.[13,14] However, no serological study has examined the association between HPV 73 and the risk of ESCC.

The effect of HPV on esophageal and gastric adenocarcinomas has not been as extensively studied. Four of the seroepidemiological studies that evaluated the association between HPV and ESCC also reported data on its association with esophageal adenocarcinomas, gastroesophageal junction adenocarcinomas, or gastric adenocarcinomas.[8,9,11,12] None of these studies found a significant association between HPV 16 and these cancers. Lagergren *et al*. found an inverse association between HPV 18 and adenocarcinomas of the esophagus or gastroesophageal junction (OR 0.2, 95% CI 0.1–0.7).

The primary goal of this prospective study was to evaluate the association between serologic markers of HPV 16, HPV 18 and HPV 73 and the risk of subsequent development of ESCC, gastric cardia adenocarcinoma (GCA) and gastric noncardia adenocarcinoma (GNCA) in a cohort of individuals from the general population of Linxian, China, an area with extremely high rates of these cancers.[15] Type specific antibodies were assessed by validated ELISA assays that were identical or similar to those used in previous serologic studies. We examined the association between HPV and these cancers using the typical binary exposure classification (exposed *vs*. unexposed), as well as, a continuous exposure metric-based on the actual magnitude of the observed optical density measurements. To investigate the sensitivity of the inference to the choice of cutpoints, we used 2 methods to define the exposure: the *standard cutpoint* which, in previous studies, has been shown

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to distinguish HPV 16-infected women from noninfected women,[16] and a *spline cutpoint*, which was chosen based on a graphical approach that utilizes the relationship of OD with risk in the study population.

In addition to estimating the risks associated with the 3 individual cancers (ESCC, GCA and GNCA), we also estimated the risk for the combined endpoint of ESCC and GCA (ESCC/GCA). Both ESCC and GCA occur at extremely high rates in the Linxian population, and ESCC/GCA has been the principal cancer endpoint since we began studying this cohort in 1985.[17,18] In recent studies of serum nutrients, we have found similar associations for these nutrients with ESSC and GCA, which differed from their associations with GNCA.[19,20]

Participants and methods

Study design

Subjects of this study were selected from the cohort of all participants in the Linxian General Population Trial. Elsewhere we have given a detailed description of the design, choice of intervention agents, methods of conduct and primary endpoint analyses of this trial.[15,17] In brief, the participants were 29,584 healthy adults aged 40–69 years from 4 Linxian communes. In the spring of 1985, 1 year prior to the start of intervention, each participant was interviewed and subjected to a brief physical examination. After collecting 10 ml of blood from the participants, serum samples were separated, aliquots were prepared and stored under frozen condition for future analyses. In accord with a partial factorial design the participants were randomly assigned to take either a vitamin/mineral combination or a placebo. The 4 different vitamin/mineral combinations tested were: factor A, retinol and zinc; factor B, riboflavin and niacin; factor C, ascorbic acid and molybdenum; factor D, β carotene, α tocopherol and selenium. Local health care providers recorded cancer incidence and mortality data at monthly intervals throughout the intervention period. Periodic surveys were conducted to verify completeness and accuracy of the medical information. Outcomes for the present study were based on follow-up data through May 1991. Diagnostic materials for 90% of the cancer cases, in this study, were reviewed by a panel of American and Chinese experts. For anatomic localization of gastric adenocarcinomas, cancers were defined as cardia cancers if they were in the most proximal 3-cm of the stomach, and noncardia cancers if originating outside this region. Ninety-five percent of anatomic localizations were made using endoscopy, surgery and/or X-rays. For cancer cases without diagnostic material and for deaths due to causes other than cancer, reviews were performed by senior Chinese experts.

The conduct of the Linxian General Population Trial was approved by the institutional review boards of the Cancer Institute of Chinese Academy of Medical Sciences and the US National Cancer Institute.

Case and control subjects

By May 1991 (after 5.25 years of intervention) there were 640 incident cases of ESCC, and 435 incident cases of GCA. Of these we selected an age- and sex-stratified random sample of 100 cases of ESCC and 100 cases of GCA for HPV serotype measurements. The age strata were defined by age groups $\leq$50 years, 51–60 years and >60 years. Among the ESCC patients, 1 patient did not have adequate serum sample for measurement of HPV antibodies, so 99 patients with ESCC were used for these analyses. Of a total of 104 incident cases of GNCA that occurred during the 5.25 years of follow-up, we included all 70 cases with adequate serum for analysis. For controls we selected a stratified random sample of 400 of the 26,951 subjects who were alive and cancer free at the end of the General Population Trial. These were sex and age frequency-matched to the ESCC and GCA cases. Of these 400 subjects, 381 had serum samples adequate for the measurement of HPV.

Assuming a 2-sided test, $\alpha = 0.05$, and 10% seropositivity in controls, 100 cases and 380 controls provided an 85% power to detect ORs of 2.5.

Serologic assays

Type-specific HPV antibodies were measured using ELISA assays for detecting antibodies to baculovirus-derived capsids containing both L1 and L2 proteins. The ELISA method used in this study has been well-established and validated previously[5,16]; this method is identical or similar to the methods used in the 5 previous studies that examined the association of HPV seropositivity and the risk of ESCC. The ELISA reactions were performed on plates with 96 wells. A total of 16 plates were required for each HPV type. All subjects had 2 measurements for each type; each measurement was on a different plate. In addition to the subjects' sera, each plate contained 4 to 6 (an average of 5.6) replicates of a *standard-control* serum, and 8 to 10 (average of 9.1) replicates of a *study-control* serum. The *standard-control* serum came from a pooled human sample [Life Technologies (GIBCO BRL), Gaithersburg, MD] and had an optical density in the ELISA test for HPV 16 virus-like particles that was equal to the optical density of the 97th percentile of women who were HPV 16-negative.[16] The *study-control* serum came from pooled serum from Linxian. These *study-control* serum replicates were randomly mixed among the samples from subjects in the study; individuals performing the laboratory assays were not aware of the presence of these controls.

Statistical methods

To test differences between subject attributes by outcome, we compared means (age) and proportions (sex, smoking, alcohol consumption) using *t*-test and χ^2-tests, respectively. All *p*-values reported are 2-sided.

To test and adjust for plate variability, and to estimate the components of within and between plate variation, we fit a random effects model to the plate controls using the statistical package developed by the SAS Institute (Cary, NC). In particular we fit $Y_{ijk} = X_i + \beta_j + \varepsilon_{ijk}$; here Y_{ijk} is the natural logarithm of the measured optical density (OD) for the *k*th measurement of control serum on plate j; X_i is the true and unobserved log OD;β_j is the effect of plate *j*; and ε_{ijk} is the within plate error for each sample. The log transformation was chosen because it resulted in ε_{ijk} which were normally distributed, homoscedastic, and uncorrelated with β_j. In the quality control samples, variance due to plate variation was 0.01 for HPV 16 and HPV 73, and 0.02 for HPV 18. Within plate variance was 0.01 for HPV 16 and HPV 73 and 0.05 for HPV 18. For all serotypes the variation due to plate was significant at $p < 0.05$. We obtained the *plate-adjusted* log OD by subtracting the estimate of the plate effect, β_j, from the subject's observed log OD. To obtain an overall measure of the subject's exposure to the type specific HPV we averaged the 2 plate-adjusted values. Subsequently, we refer to this measure as the subject's log OD. This procedure of adjusting for plate effect is conceptually equivalent to the usual procedure of calculating a subject's optical density by averaging the ratios of an individual's plate-specific optical density to the average of standard controls on the plate. The main mathematical differences between the 2 methods are that the usual procedure does not utilize information from the study-controls to adjust for plate effects, and it does not provide estimates of the magnitude or the significance of the components of variability.

Based on this log OD and a threshold cutpoint value, *C*, we classified exposure to HPV into both a categorical and continuous measure. For all the risk estimates presented in the tables of this paper (Tables II–III) *C* was defined to be the average log OD of the standard-serum plate controls. The log OD values for these cutpoints were −0.23, −0.68 and −0.63 for HPV types 16, 18 and 73, respectively. For the categorical analyses we classified individuals as exposed if and only if their log OD was greater than

TABLE I – DEMOGRAPHIC CHARACTERISTICS, TOBACCO USE AND ALCOHOL CONSUMPTION AMONG CASES AND CONTROLS

	Controls (n = 381)	ESCC (n = 99)	GCA (n = 100)	GNCA (n = 70)
Mean age ± SD (yr)	55.0 ± 9.0	54.8 ± 8.5	56.1 ± 8.8	58.3 ± 7.4
Number of males	187 (49)	49 (49)	50 (50)	52 (74)
Number of male smokers[1]	118 (63)	38 (78)	39 (78)	36 (69)
Number of alcohol consumers[1]	83 (22)	25 (25)	22 (22)	17 (24)

Values in parentheses indicate percentages

[1]Smoking and drinking were both categorized as binary variables. Smoking in this population was almost entirely limited to male subjects, and so the numbers and percentages were calculated only for men. Male subjects who ever smoked cigarettes for 6 or more months were classified as smokers; subjects who drank any alcoholic beverage in the last 12 months were classified as alcohol consumers.

TABLE II – NUMBERS AND UNADJUSTED ODDS RATIOS FOR SEROPOSITIVITY TO EACH HPV TYPE AMONG CASES AND CONTROLS[1]

	HPV 16		HPV 18		HPV 73	
	N	OR	*N*	OR	*N*	OR
Controls	29 (8)	1.0	35 (9)	1.0	42 (11)	1.0
ESCC	12 (12)	1.7 [0.7–3.5]	8 (8)	0.9 [0.3–2.0]	13 (13)	1.2 [0.6–2.5]
GCA	10 (10)	1.3 [0.6–3.0]	8 (8)	0.9 [0.3–2.0]	13 (13)	1.2 [0.6–2.4]
GNCA	3 (4)	0.5 [0.1–1.8]	8 (11)	1.3 [0.5–3.0]	8 (11)	1.0 [0.4–2.4]

Values in parentheses indicate percentages. Values in square brackets indicate 95% CI.

[1]The cutpoint used for this analysis was two standard deviations above mean for a population who had 0 or 1 sexual partners and tested negative for cervical HPV DNA.

C. For the continuous classification those subjects whose log OD was less than or equal to C were given an exposure value of 0; for the other subjects, the continuous exposure value was based on the subject's log OD minus the cutpoint value C. Specifically, if we let Y_i be the log OD for subject i, then the continuous exposure is calculated as, $(Y_i - C)/0.2$ when $Y_i > C$; and $Y_i = 0$, when $Y_i \leq C$. The standardizing unit of 0.2 was chosen because it is approximately equal to the difference between the 75th and 25th percentile log OD for the controls for each HPV type.

In addition to the *standard cutpoints*, we estimated cutpoints based on cubic spline logistic regressions of the OR of ESCC, GCA, ESCC/GCA and GNCA versus the magnitude of the type-specific log OD. We considered for cutpoints any inflection point in the graph of OR versus log OD. Here the inflection points were visually defined to be the value of the log OD beyond which the OR either monotonically increased or decreased. We designated the *spline cutpoint* for each HPV type as the smallest such inflection point for any cancer outcome. For HPV 16 and HPV 73 there were points of inflection beyond which the risk of ESCC and GCA seemed to increase with increasing log OD; there was no relation of risk and these serotypes for GNCA. Using the ESCC/GCA spline regression for HPV 16 the *spline cutpoint* was indistinguishable from the *standard cutpoint* of −0.23. For HPV 73 the point of inflection occurred at −0.80. For HPV 18 there was no relation of risk to either ESCC or GCA. Based on the cubic spline regression of HPV 18 and GNCA we chose a *spline cutpoint* of −0.80.

Logistic regression models were fit to estimate the increase in odds associated with HPV type separately for each of the cancer endpoints ESCC, GCA, ESSC/GCA and GNCA. We made estimates for both the categorical and continuous exposure metrics using both the standard and *spline cutpoints*. The adjusted ORs were estimated in models that include terms for age (in years), sex, smoking and drinking. Ninety-five percent confidence intervals (CI)were formed using the Wald procedure; p-values comparing nested models are from likelihood ratio tests and are 2-sided. Estimating risk in logistic models wherein all HPV serotypes were simultaneously included did not substantially alter ORs or confidence intervals. Hence these estimates are not given.

The risk estimates did not change with adjustment for the treatments used in the Linxian General Population Trial, and treatment assignment was independent of HPV status.

Results

Table I summarizes the demographic characteristics, smoking status and alcohol consumption status of the cases and controls of this study. By design, controls were frequency matched to ESCC/GCA cases for age and sex. GNCA cases, however, were more common in male subjects than in control subjects (75% *vs*. 49%; $\chi^2 = 15.08$, $p < 0.001$). Smoking was more frequent among male ESCC cases (78%) and male GCA cases (78%) than in male controls (63%) ($\chi^2 = 3.46$, $p = 0.06$ and $\chi^2 = 3.75$, $p = 0.05$, respectively), but the difference between smoking in GNCA cases and controls was not significant ($\chi^2 = 0.60$, $p = 0.44$). Alcohol consumption was similar in all groups. The frequencies of smoking and alcohol consumption by case status were similar to those previously reported for the full cohort.[21]

To evaluate the importance of the OD threshold used for categorization into HPV exposed and unexposed, we used both a *standard cutpoint*, based on the 97th percentile for OD in women who had 0 or 1 sexual partners and tested negative for cervical HPV DNA,[16] and a *spline cutpoint*, based on a graphical analysis of how cancer risk varied with the OD measurement (see methods). For HPV 16 this cutpoint was virtually identical to the *standard cutpoint*, so no new spline threshold was defined. For HPV 18 and HPV 73 the *spline cutpoints* were lower than the *standard cutpoints*. Using the *spline cutpoints* increased the control exposure prevalances of 9% (HPV 18) and 11% (HPV 73), based on the *standard cutpoints*, to prevalances of 18% (HPV 18) and 19% (HPV 73). Despite these differences in exposure classification, the risk estimates were nearly identical for both sets of cutpoints. Hence we present estimates only for the *standard cutpoints*.

Table II shows the proportion of seropositive cases and controls for each type of HPV, and the unadjusted ORs and 95% confidence intervals, for the binary exposure classification (exposed *vs*. unexposed). All ORs were close to 1 (ranging from 0.5 to 1.7) and all CI included 1.

Table III, which also includes the outcome ESCC/GCA, gives the adjusted OR's using both the binary and continuous measures of HPV exposure. For the binary classification, adjustment produced virtually no change in the risk estimates for any cancer site or HPV type. For the continuous metric, the given OR's show the risk associated for each increment of 0.2 in log OD. This unit was approximately equal to the difference between 75th and 25th per-

TABLE III – ADJUSTED[1] ODDS RATIOS FOR THE ASSOCIATION BETWEEN EACH TYPE OF CANCER AND CATEGORICAL AND CONTINUOUS HPV OUTCOMES

	HPV 16		HPV 18		HPV 73	
	Categorical OR[2]	Continuous OR[3]	Categorical OR	Continuous OR	Categorical OR	Continuous OR
ESCC	1.6 (0.8–3.3)	1.3 (0.8–2.2) [0.32]	1.0 (0.4–2.2)	1.4 (0.7–2.7) [0.40]	1.3 (0.6–2.5)	1.4 (0.9–2.1) [0.12]
GCA	1.3 (0.6–2.8)	1.2 (0.7–2.1) [0.52]	0.9 (0.4–2.1)	1.5 (0.8–2.7) [0.26]	1.2 (0.6–2.3)	1.4 (0.9–2.1) [0.15]
ESCC/GCA	1.5 (0.8–2.7)	1.3 (0.8–2.0) [0.28]	0.9 (0.5–1.8)	1.4 (0.8–2.4) [0.22]	1.2 (0.7–2.1)	1.4 (1.0–1.9) [0.07]
GNCA	0.4 (0.1–1.6)	0.8 (0.3–1.8) [0.50]	1.5 (0.6–3.4)	1.2 (0.5–2.8) [0.71]	0.9 (0.4–2.1)	1.0 (0.6–1.9) [0.97]

Values in parentheses indicate 95% CI. Values in square brackets indicate *p* for trend.
[1]Adjusted for age, sex, smoking and alcohol consumption.–[2]The cutpoint used for categorical analysis was two standard deviations above mean for a population that had 0 or 1 sexual partners and tested negative for cervical HPV DNA.–[3]Each unit was an increment of 0.2 in log OD, which was approximately equal to the difference between the 75th and 25th percentiles of log OD in the controls.

TABLE IV – SEROLOGIC STUDIES OF HPV 16 AND HPV 18 AND ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Study	Design	Study location	Population ESCC risk	Cases/ Controls	HPV 16			HPV 18		
					Cases, *N*	Controls, *N*	OR	Cases, *N*	Controls, *N*	OR
Dillner[8a]	Prospective	Finland	Low	29/78	7 (24)	2 (3)	12 [2.0–123]	–	–	–
Bjorge[9]	Prospective	Norway	Low	41/123	4 (10)	2 (2)	10 [1.0–510]	6 (15)	9 (7)	2.1 [0.6–6.9]
Han[10]	Retrospective	China	High	90/121	22 (24)	6 (7)	4.5 [1.8–11.9]	–	–	–
Lagergren[11]	Retrospective	Sweden	Low	121/302	14 (12)	33 (11)	1.0 [0.5–2.0]	6 (5)	29 (10)	0.5 [0.2–1.1]
Van Doornum[12a]	Retrospective	Netherlands	Low	56/100	8 (14)	18 (18)	0.8 [0.3–2.0]	–	–	–
Current study	Prospective	China	High	99/389	12 (12)	29 (8)	1.6 [0.8–3.3]	8 (8)	35 (9)	1.0 [0.4–2.2]

Values in parentheses indicate percentages. Values in square brackets indicate 95% CI.
[a]Unconditional logistic regression was used to calculate ORs, using numbers presented in the papers.

centile of log OD in the controls. The binary and continuous analyses both showed a slight but statistically nonsignificant increase in risk of ESCC and GCA associated with HPV 16 and HPV 73. For the combined ESCC/GCA endpoint, the binary classification showed a 50% increased risk for HPV 16 (OR 1.5, 95% CI 0.8–2.0); for HPV 73 the increase was 20% (OR 1.2, 95% CI 0.7–2.1). For GNCA, there was a nonsignificant reduction in risk with HPV 16 (OR 0.4, 95% CI 0.1–1.6) and a nonsignificant increase in risk with HPV 18 (OR 1.5, 95% CI 0.6–3.4).

Subjects who were seropositive for any 1 HPV type were more likely than the seronegative subjects to be seropositive for each of the 2 other types. When compared with the HPV 16 seronegative subjects, the seropositive subjects had ~2 (OR = 2.2, $p = 0.04$) or 3 (OR = 3.4, $p < 0.001$) times the risk of being seropositive for HPV 18 and HPV 73, respectively. Subjects seropositive for HPV 18 were 3 times as likely (3.0, $p < 0.001$) as the seronegative subjects to test positive for HPV 73. Including all 3 serotypes in a single logistic model of cancer produced no noteworthy changes in the risks estimates given in Tables II and III (data not shown).

Discussion

Since 1982, when a role for HPV in the etiology of ESCC was first suggested, multiple epidemiologic studies have used a variety of techniques to assess the association between HPV infection and the risk of ESCC.[4] Some of these techniques (*e.g.*, filter *in situ* hybridization) were later shown to have low sensitivity and poor specificity, and the results of the studies using those techniques are not considered reliable.[4,22] HPV DNA studies using polymerase chain reaction and type-specific serologic studies seem to be the most accurate methods to detect exposure to HPV in epidemiologic studies. Unfortunately, the results of PCR studies have not been consistent: these studies have found evidence for presence of HPV in the tumor tissues varying from 0 to 67%.[4] A review shows that most PCR studies showing a high prevalence of HPV in esophageal tumors have been conducted in areas with very high rates of ESCC.[4] By contrast, most studies that have failed to show HPV in the tumors have been conducted in areas with low risk of ESCC.

HPV type-restricted serologic tests were first developed in the mid-1990s and were shown to be relatively specific. Virus-like particles used for serologic detection of HPV 16 were shown to react with IgG antibodies in 59% of women who tested positive for cervical HPV 16 DNA, but with IgG antibodies of only 6% of women who tested negative.[5] Serologic tests have been found to be stable over time, even after 1 decade of follow-up,[23] and they correlate well with the life-time number of sexual partners.[7,24,25] Therefore, HPV serologic tests are considered good markers of long-term exposure to HPV, and are probably useful to detect an association between HPV and ESCC even if HPV has a ''hit-and-run'' mechanism for inducing esophageal cancer, a phenomenon that has been seen in cancers of the bovine foregut induced by bovine papillomavirus.[26]

Five previous studies have used serologic methods to examine the association between HPV 16 and the risk of ESCC (Table IV). The 2 prospective studies, both conducted in Europe, found very strong associations between seropositivity to HPV 16 and the risk of ESCC: Dillner *et al*. found an OR (95% CI) of 12 (2.0–123),[8] and Bjorge *et al*. found an OR of 10 (1.0–510) for this association.[9] A retrospective (case-control) study in China by Han *et al*. also found a strong association between HPV 16 and ESCC, with an OR of 4.5 (1.8–11.9).[10] In contrast, the other 2 retrospective studies, both conducted in Europe, found no association between HPV 16 and the risk of ESCC: Lagergren *et al*. found an OR of 1.0 (0.5–2.0), and Van Doornum *et al*. found an OR of 0.8 (0.3–2.0).[11,12] Four of these serologic studies also examined the association between HPV 16 and the risk of esophageal or gastric adenocarcinoma (Table V). None of these studies found a significant association between HPV 16 and these cancers.

The current study is the largest prospective serologic study that has assessed the association of HPV 16 and risk of ESCC (99 cases), GCA (100 cases) or GNCA (70 cases). We found no significant associations between HPV 16 seropositivity and cancer risk at any of these sites. As with some of our previous observational studies in this population,[17,19,20] the associations with HPV 16 were similar for ESCC, with an OR of 1.6 (0.8–3.3), and GCA, with an OR of 1.3 (0.6–2.8), and were different for GNCA, with an OR of 0.4 (0.1–1.6).

There are several mechanisms that may contribute to the differences observed in the association of HPV 16 and ESCC in serologic studies. The most likely include the methods of choosing the OD cutpoint that defines seropositivity, differences in what characteristics (*e.g.*, smoking status and alcohol consumption) are included in the adjusted estimates, chance fluctuation due to the small number of cases in some studies, study design (prospective *vs*. retrospective) and geographic variation.

TABLE V – SEROLOGIC STUDIES OF HPV 16 AND HPV 18 AND ESOPHAGEAL OR GASTRIC ADENOCARCINOMA

Study	Design	Study location	Cancer site	Cases/ Controls	HPV 16			HPV 18		
					Cases, *N*	Controls, *N*	OR	Cases, *N*	Controls, *N*	OR
Dillner[8a]	Prospective	Finland	EAC[b]	10/78	1 (10)	2 (3)	4.2 [0.06–86]	–	–	–
Bjorge[9a]	Prospective	Norway	EAC[b]	16/48	1 (6)	2 (4)	1.5 [0.02–31]	1 (6)	1 (2)	3.1 [0.04–251]
Lagergren[11]	Retrospective	Sweden	E+GEJA	173/302	21 (12)	33 (11)	1.2 [0.7–2.2]	4 (2)	29 (10)	0.2 [0.1–0.7]
Van Doornum[12a]	Retrospective	Netherlands	EAC	48/100	11 (23)	18 (18)	1.4 [0.5–3.4]	–	–	–
			Stomach	54/100	10 (18)	18 (18)	1.0 [0.4–2.6]	–	–	–
Current Study	Prospective	China	GCA	100/381	10 (10)	29 (8)	1.3 [0.6–2.8]	8 (8)	35 (9)	0.9 [0.4–2.1]
			GNCA	70/381	3 (4)	29 (8)	0.4 [0.1–1.6]	8 (11)	35 (9)	1.5 [0.6–3.4]

Values in parentheses indicate percentages. Values in square brackets indicate 95% CI. E+GEJA, esophageal + gastroesophageal junction adenocarcinoma; EAC, esophageal adenocarcinoma; GCA, gastric cardia adenocarcinoma; GNCA, gastric noncardia adenocarcinoma.

[a]Unconditional logistic regression was used to calculate ORs, using numbers presented in the papers.–[b]EAC in these studies refers to esophageal cancers other than esophageal squamous cell carcinoma.

Establishing the most appropriate cutpoint for seropositivity to HPV is an issue of potential concern. Some of the previous studies found considerable variation in the association between HPV 16 exposure and the risk of ESCC when they changed their cutpoints.[9,10] For example, in Bjorge's study[9] the OR for the association of HPV 16 and ESCC changed from 10 for a cutpoint of 0.239 (a level that that had given optimal discrimination of cases and controls in a previous study[27]) to 2.3 for a cutpoint of 0.100 (a level that distinguishes HPV 16 infected women from sexually inexperienced women[28]). To overcome this problem, we examined graphs of risk versus type-specific log OD produced by cubic spline logistic regressions and defined a *spline cutpoint* as the inflection point beyond which the graph either monotonically increased or decreased. We also used *standard cutpoints*, which were cutpoints used in prior studies. In our study, these 2 cutpoints produced very similar results.

Some of the previous studies have failed to adjust for smoking status or alcohol consumption,[8–10] but in 1 study that did adjust for both of these potential confounders, this adjustment did not significantly affect the results.[11] Adjustment for age, sex, smoking status and alcohol consumption did not change the results of our study either.

The small size of some of the previous studies explains part of the inconsistency of results found in these studies. For example, Dillner *et al*.[8] had only 29 cases of ESCC, so their 95% CI ranged from 2.0 to 123. In addition, the controls matched to esophageal cancer cases in this study had a much lower prevalence of HPV 16 than all controls in the study (3% *vs*. 8%), which probably led to an inflated estimate of the risk ratio. The risk ratio confidence intervals for the association of HPV 16 and ESCC in the other previous studies were 1.8–11.9,[10] 1.0–510,[9] 0.5–2.0[11] and 0.3–2.0.[12] Several of these CIs are very wide, indicating unstable risk estimates. All of these CIs overlap those obtained in our study (0.8–3.3).

Another possible reason for the different results seen in the serologic studies of HPV exposure and ESCC risk is the geographic variation in this association. HPV DNA is more commonly found in ESCC tumors from areas with a high incidence of ESCC,[4] and this suggests that the association between HPV and ESCC may be stronger in these areas. But this has not been the case in the serologic studies of HPV 16 and ESCC, in which the strongest associations have been reported from low-risk European countries and studies in both high- and low-risk populations have shown discrepant results. The 2 studies that found the highest OR's for the HPV 16-ESCC association were conducted in Finland[8] and Norway[9] but similar studies in Sweden[11] and the Netherlands[12] found null results. The 2 studies from high-risk areas of China also produced inconsistent results. The previous study from Shaanxi[10] found a positive association, while our study from Linxian did not. One difference between these 2 Chinese populations is that cervical cancer is much more common in Shaanxi, raising the possibility that there might be a higher prevalence of oncogenic HPV types in this area, but the HPV 16 seropositivity in the controls in these studies was essentially identical (7% *vs*. 8%, respectively). Thus, both strong and null associations have been reported in serologic studies from both Europe and China, and from both high- and low-risk areas for ESCC, so at this point there appears to be no consistent relationship between geography or population ESCC risk and HPV 16 serologic study results.

Only 2 previous serologic studies, 1 prospective and 1 retrospective, have examined the association between HPV 18 and esophageal or gastric cancers. The OR's found in these studies for ESCC were 2.1 (0.6–6.9) and 0.5 (0.2–1.1), and the numbers for esophageal or gastroesophageal junction adenocarcinomas were 3.1 (0.4–251) and 0.2 (0.1–0.7), respectively.[9,11] In our study, we found no significant association between HPV 18 and ESCC (OR = 1.0; 0.4–2.2), GCA (OR = 0.9; 0.4–2.1) or GNCA (OR = 1.5; 0.6–3.4).

HPV 73 has been reported to be the most common type of HPV found in benign and malignant esophageal tumors,[13,14] but has not been classified as human carcinogen during recent IARC assessment.[29] This is the first study to test for an association between a serologic marker for HPV 73 and the risk of upper gastrointestinal cancer. We found no significant association between HPV 73 and ESCC, GCA or GNCA. We acknowledge that the associations in this study were estimated with limited precision. However, the confidence intervals suggest that the association between HPV 16 and ESCC in Linxian, if it exists, is unlikely to exceed 2-fold.

In summary, using validated serologic tests, we prospectively examined potential associations between HPV 16, HPV 18 and HPV 73 and the occurrence of upper gastrointestinal cancers in Linxian, China, an area with extremely high rates of these cancers. The results of this study do not support a major role for these HPV types in the etiology of esophageal and gastric cancers in Linxian.

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